The Comparative Impact of Acesulfame-Potassium, Stevia Extract, and Monk Fruit on the Lifespan of Drosophila melanogaster

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Non-nutritive sweeteners (NNS), non-caloric sweetening devices, have been widely commercialized to reduce sugar consumption. This intent has associated NNS with health benefits, though reports have correlated the consumption of these substitutes with noncommunicable diseases. The interaction between NNS and distinct physiological processes has been examined, though research into the broader implications of these interactions, toward longevity, for instance, is lacking. The purpose of this study was to compare the impact of three recently FDA-approved NNS - acesulfame-Potassium (ace-K), stevia, and monk fruit - on the survival of Drosophila melanogaster, a model species for longevity research, to discern the possible impacts on human longevity, and further, the rationality of their use. It was hypothesized that if D. melanogaster were fed these three NNS, those fed with ace-K would have the lowest survivorship rates, as ace-K is associated with microbial dysbiosis, which is further linked to reduced longevity. Fifteen male and female age-synchronized flies were allocated to vials containing a sweetener incorporated into a base diet, with sucrose as the control. Survivorship was recorded every three to four days for 32 days. Survival from each diet was significantly lower than the control, and this difference was most pronounced with ace-K, $\chi^2 (9, N=240)=244.2, p < .00001$. Survivorship between the diets halfway through the trial period (day 15) was also significantly different, $\chi^2 (3, N=240)=78.3, p < .00001$. Ace-K, therefore, had a detrimental effect on the longevity of D. melanogaster, which suggests a potential paralleled effect of this sweetener in humans.

Introduction

An unhealthy diet is the chief risk factor for morbidity in the United States and, in 2017, 11 million deaths internationally were the result of poor nutrition factors, such as consumption of calorically-dense foods. Glucose control, insulin response, and metabolism - which have long-term health implications - are heavily influenced by dietary choices. The prevalence of sugar consumption, for instance, has been linked to the development of metabolic syndrome, encompassing type 2 diabetes, obesity, hypertension, and cardiovascular disease. Sugar intake can also lead to chronic inflammation via the production of inflammatory cytokines, which can further contribute to increased susceptibility for noncommunicable diseases. In 2017-2018, Americans consumed an average of 17 teaspoons of added sugar every day, which surpassed the maximum intake recommendations provided by the World Health Organization.

To address this issue, non-nutritive sweeteners (NNS), also known as sugar substitutes, have been developed to curb the consumption of caloric sugar. These substitutes, now widely commercialized in “diet” and “sugar-free” products, are typically non-caloric, meant to pass through the body unmetabolized, and have no glycemic effects, while still offering consumers a sweet taste. Thus, as NNS are designed to reduce caloric excess, high blood pressure, and inflammation, they are widely thought to be healthier alternatives to traditional sugar. Contrary to this standard assumption, epidemiological findings have presented a correlation between the intake of NNS and the development of noncommunicable diseases, such as type 2 diabetes, obesity, and metabolic syndrome. NNS may also alter the gut microbiome, a complex community of bacteria that play a vital role in metabolism and immune response, which can further contribute to inflammation and chronic disease. Six NNS have been approved by the FDA, one of the most recent being acesulfame-Potassium (ace-K). Two have been “Generally Recognized as Safe” (GRAS) - stevia and monk fruit.

Ace-K was approved by the FDA in 2003 with an Acceptable Daily Intake (ADI) of 15 mg/kg of body weight and a sweetness 200 times that of sucrose. Ace-K, part of the oxathiazinonodioxide class, is artificially manufactured from potassium and an asulfame salt. Ace-K is quickly taken up by the body and excreted in urine.

Deemed GRAS in 2007, stevia, coming from the Stevia rebaudiana leaf, gets its natural non-nutritive sweetness from steviol glycosides, the most plentiful being stevioside and rebaudioside A. Steviol glycosides are metabolized to steviol, which is then absorbed and excreted through urine. Stevia has an ADI of 4 mg/kg of body weight.

Monk fruit extract, also known as Lu Han Guo or Siraitia grosvenorii, is a natural NNS that was considered GRAS, though without an ADI, in 2010. Mogrosides, specifically mogroside V, are a type of incompletely absorbed glycoside significant to monk fruit’s composition and are about 300 times sweeter than sucrose. Digestive enzymes in the colon and bacteria in the microbiome metabolize monk fruit, which is excreted in the feces.

Assessments of NNS have utilized various model organisms, including Drosophila melanogaster, the fruit fly, which is widely accepted for studying human metabolism due to the conservation of metabolic pathways, genetic similarities, and their simple, though significant, microbial communities. D. melanogaster also have short lifespans of just four to six weeks.

Research in other organisms, including mice, has revealed that the consumption of ace-K stimulates weight gain and increases the concentration of intestinal Bacteroides, which are associated with obesity, in a dose-dependent manner. Ace-K also increases genes involved in lipopolysaccharide synthesis, a phenomena directly related to inflammatory responses, and perturbes the fecal matter of mice, which is evidence of microbial imbalance, or dysbiosis, that may impact glucose homeostasis. Conflicting results have been reported, though, with smaller doses of ace-K resulting in neutral microbial, glucose, and insulin responses.

Stevia is non-toxic and has a neutral short term effect on glucose and insulin responses in diabetic patients. Stevioside glycosides have antioxidant and antidiabetic attributes, although, similar to ace-K, stevioside and rebaudioside A hinder the growth of anaerobic and aerobic bacteria, respectively.

In addition, stevia increases the frequency of short chain fatty acids (SCFAs), which have a variety of health benefits, produced by the microbiome.

Monk fruit has not been observed to impact glucose control, although mogrosides have been reported to alter microbial populations in a lab setting. Monk fruit has a neutral impact on the development and reproduction of mammals, although its genotoxicity has not been determined.
The sweetener has also been associated with anti-diabetic, anti-inflammatory, anti-oxidant, and anti-tumor properties.7
The gut microbiome has emerged as an interest in nutritional analysis as dysbiosis can result in altered glucose homeostasis and age-related morbidities.14 Decreased commensal bacteria is directly related to a reduction of healthful SCFAs, which protect the body against chronic inflammation.14 Healthy aging, therefore, is dependent upon the microbiome to prevent the release of pro-inflammatory mediators, the inhibition of beneficial microbes, and disrupted microbial metabolism, all of which can decrease longevity.14 Ace-K, stevia, and monk fruit induce dysbiosis, so a causal relationship may exist between these sweeteners and lifespan.4,9,13 However, longevity research has not been conducted for these NNS.14

The purpose of this study, therefore, was to determine the impact of recently approved NNS on the longevity of Drosophila melanogaster to further prompt research and reconsideration into the commercialized usage of these substances in regard to human aging. Survival rates in this study were analyzed to propound parallel outcomes for humans, which were then intended to enhance the public health understanding of sugar substitutes. Results of the study may address the potential impact of NNS on the development of metabolic disorders, chronic inflammation, and noncommunicable diseases, all of which may decrease lifespan. It was hypothesized that if D. melanogaster were fed ace-K, stevia extract, and monk fruit, the consumption of ace-K would result in the lowest survivorship. This postulate was justified by ace-K’s tendency to induce microbial dysbiosis, which is linked to inflammation and, further, decreased longevity.4,14 Two groups of vials containing 30 age-synchronized D. melanogaster each were fed diets of equal concentrations (g/L) of ace-K, stevia, or monk fruit, as well as the control group of caloric sugar (sucrose). Death counts were taken every three to four days when flies were moved to fresh food, and longevity was measured as number of survivors. Data were analyzed using the Chi-Square Goodness of Fit test.

**Methods**

To prepare fly media, 15 g of agar powder was mixed with 700 mL of distilled water in either a 1000 mL volumetric or Erlenmeyer flask and then boiled on a hot plate. 100 g of Fleischmann’s instant dry yeast was mixed into the solution. 50 g of either ace-K, stevia, monk fruit, or Domino’s sugar were also mixed in. Water was added so that each sweetener’s concentration was 50 g/L (Figure 1). The solution was allowed to cool at room temperature while being covered withparafilm to avoid external contamination. The flask was re-heated to allow approximately two centimeters of media to be poured into two sterile 50 mL glass vials. A Sharpie and label tape were used to label the vials with the name of the sweetener included and vial number. Vials were then plugged with fitted cotton tops and transferred to an approximately 4° Celsius refrigerator. This media preparation procedure was repeated for each sweetener, with two vials being used per sweetener. Remaining media not immediately allocated to vials were stored in covered 150 mL beakers in a refrigerator for later use. Before and after preparation, the counter top and all materials were wiped with a Lysol wipe. Masses of solutes were taken using weigh boats and electronic balances.
Three vials of Drosophila melanogaster were put on their side in a freezer for approximately five minutes until adult flies were anesthetized. The adult flies were then transferred into a 91% isopropyl alcohol solution for termination, which was then disposed of in a biohazardous waste container. The original vials were re-plugged and left undisturbed until remaining pupae emerged. Upon the emergence of experimental pupae, the flies were anesthetized following the previous procedure and sorted into eight groups of 15 males and eight groups of 15 females with a sorting brush on an ice pack sterilized with a Lysol wipe. One group of each sex was transferred to each vial with a paint brush so that there were 30 flies in total and 15 flies of each sex per vial, with two vials for each diet (Figure 1). Vials were securely plugged with fitted, breathable plugs. Remaining flies were left in vials and euthanized following the aforementioned procedure.
Every three to four days, deaths were counted by observing motionless flies, and upon anesthetizing, surviving D. melanogaster were transferred to new vials with fresh media made with the same sweetener the respective group of flies had begun with. Old media was heated to 100°C on a hot plate. 100 g of Fleischmann’s instant dry yeast was mixed into the solution. 15 g of agar powder was mixed with 700 mL of distilled water in either a 1000 mL volumetric or Erlenmeyer flask and then boiled on a hot plate. 100 g of Fleischmann’s instant dry yeast was mixed into the solution. 50 g of either ace-K, stevia, monk fruit, or Domino’s sugar were also mixed in. Water was added so that each sweetener’s concentration was 50 g/L (Figure 1). The solution was allowed to cool at room temperature while being covered with parafilm to avoid external contamination. The flask was re-heated to allow approximately two centimeters of media to be poured into two sterile 50 mL glass vials. A Sharpie and label tape were used to label the vials with the name of the sweetener included and vial number. Vials were then plugged with fitted cotton tops and transferred to an approximately 4° Celsius refrigerator. This media preparation procedure was repeated for each sweetener, with two vials being used per sweetener. Remaining media not immediately allocated to vials were stored in covered 150 mL beakers in a refrigerator for later use. Before and after preparation, the counter top and all materials were wiped with a Lysol wipe. Masses of solutes were taken using weigh boats and electronic balances.
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**Results**

The survival of Drosophila melanogaster fed with sucrose, ace-K, stevia, and monk fruit was unsustained over the course of 32 days. There was high variability in survivorship between groups, which was most noticeable toward the middle of the experiment, such as on day 22 (M=11.5, SD=14.5), where the highest survival rate was sustained at 50% and the lowest was at 0% (Figure 2).

Each diet resulted in similar survivorship between both vials of 30 D. melanogaster. The sucrose diet resulted in the highest rate of survivorship in both vials, with more than 50% of flies surviving until day 22. The ace-K diet resulted in the lowest longevity rates, with less than 50% of flies alive on day 4 in both vials. In contrast, on the same day, D. melanogaster in the control group (sucrose) were all still alive. The stevia diet saw the highest variability between vials, although this difference was still only that of seven survivors at most. In both stevia vials, all D. melanogaster were deceased by day 18, while at least half of the flies fed on sucrose were alive at this time. Survivorship of the monk fruit diet was most similar to the sucrose diet in both vials, although it was still slightly lower each time the counts were recorded (Table 1).

D. melanogaster fed with sucrose had the highest total survival rate, as no deaths were observed until day 8, and mortality was gradual. The monk fruit-fed group had the second longest survival rates, with survivorship dipping below 50% only on day 18. Survivorship rates experienced the steadiest decline between days 15 and 25, with only slightly increased mortality between days 25 and 32. The stevia diet resulted in complete mortality of the sample by day 18. D. melanogaster that were fed with ace-K had the lowest survival rate, with an immediate sharp decline in survivorship and zero survivors by day 11. Total survivorship in the ace-K group over the course of 32 days differed significantly from the expected count (as given by the survivorship in the sucrose-fed group), χ² (9, N=240)=244.2, p < .00001. Total survivorship in the stevia-fed group, χ² (9, N=240)=167.1, p < .00001, and the monk fruit-fed group, χ² (9, N=240)=20.6, p < .05, were also each significantly different than the expected survivorship (Table 3).

Survivorship of Drosophila melanogaster decreased when measured every three to four days for each diet tested. In the middle of the experiment (day 15), the sucrose diet had the most survivors, with monk fruit, stevia, and ace-K, respectively, retaining fewer survivors (Figure 3).
Day 15, the midpoint of the trial period, was used as a benchmark to compare survivorship across diets. These counts differed significantly from each other and from the expected survivorship count (given by sucrose), $\chi^2 (3, N=240)=78.3, p < .00001$ (Table 4).

### Discussion

This study compared the impact of recently FDA approved non-nutritive sweeteners (NNS) - ace-K, stevia, and monk fruit - on the survivorship of *Drosophila melanogaster* to discern the potential drawbacks of their use as dietary substitutes for sucrose. The established utility of *D. melanogaster* as a model for human aging provided data that could be applied toward broader longevity research. It was hypothesized that the ingestion of ace-K would result in the lowest survival rates because of its disruptive interaction with the gut microbiome of other organisms. The decision to reject the null hypothesis was supported by the significantly shorter lifespans of *D. melanogaster* fed with ace-K in comparison to sucrose, $\chi^2 (9, N=240)=244.2, p < .00001$, and significantly different survivorship outcomes from the other sweeteners at the midpoint of experimentation (day 15), $\chi^2 (3, N=240)=78.3, p < .00001$. These results mean that survivorship from the ace-K diet was significantly lower than sucrose and other tested NNS.

Persistent, sustained variability in average survivorship of *D. melanogaster* across the four groups over the duration of the study underscores the necessity to consider each NNS separately. This observation is particularly imperative when considering the widespread usage of NNS and their potential impact on healthy aging.

The two vials for each diet had nearly identical survivorship counts for each diet, which indicates a strong reliability and reproducibility of the data. Sustained survivorship on the sucrose diet compared to each NNS suggests that this option was the most beneficial for the longevity of *D. melanogaster* overall. The sucrose diet also saw the highest survivorship by the end of the experiment (day 32), which helps establish sucrose as a fitting control, because the lifespan of *D. melanogaster* is typically at least four weeks. Ace-K overwhelmingly resulted in the lowest longevity rates, meaning that this NNS was the most harmful to the survivorship of *D. melanogaster*. The longest survivors in the ace-K group lived only one quarter of the species’ minimum predicted lifespan. These shortened longevity rates are most likely indicative of disruption to lifespan-related physiological processes due to the consumption of ace-K. Likewise, the notably lower survivorship of *D. melanogaster* on the stevia diet compared to the control displays the potential deleterious impacts of stevia on longevity. Further, the fact that survivorship in the stevia diet had ceased in just slightly more than two weeks, half of the minimum predicted lifespan of *D. melanogaster*, implies the occurrence of stevia-mediated biological disturbances. Survivorship counts of *D. melanogaster* fed with monk fruit remained similar to those in the sucrose diet throughout the study, which suggests that monk fruit has relatively similar overall consequences as sucrose. Still, survivorship of *D. melanogaster* fed with monk fruit was lower than those fed with sucrose, which may suggest biological disturbances upon the consumption of monk fruit.

Statistically, each NNS yielded significantly lower survival rates than sucrose, meaning that survivorship discrepancies between ace-K, stevia, and monk fruit were likely due to properties of these sweeteners. Therefore, NNS cannot be substituted for sucrose without thorough consideration of their unique health implications. The statistically significant difference between the surviviorships of each NNS in comparison to both sucrose and each other halfway through the experiment (day 15), $\chi^2 (3, N=240), 78.3, p < .00001$, further supports this need for individual consideration of NNS. Cumulatively, the statistical significance in the differences in longevity indicates that each of the tested sweeteners likely exhibited some harmful effects on the physiology of *D. melanogaster*. The fact that *D. melanogaster*’s biology can serve as a model of human aging further suggests that the ingestion of the NNS may yield similar physiological reactions in higher-order species.

The deleterious effect of ace-K on the longevity of *D. melanogaster* suggests a similar impact on the organism’s lifespan-modulating processes. One possible example of this physiological obstruction was given in a 2017 study showing that the ingestion of ace-K (37.5 mg/kg/day) resulted in microbial dysbiosis in male mice, as seen via a dramatic increase in *Bacteroides* and subtle shifts in other populations within the gut. Ace-K’s impact on the gut may serve to explain the poor survivorship associated with the ace-K diet because microbial dysbiosis disrupts the host’s immune system, leading to chronic inflammation, which can decrease longevity. Additionally, *Bacteroides*, although typically beneficial when in normal population levels in the gut, have pathogenic properties in other areas of the body. Stevioloside, another main component of the sweetener, decreased the growth of anaerobic bacteria, which was not the case in this study’s experiment. An explanation of the observed harmful effects of stevia may be found through consideration of the gut microbiome, as research has reported that rebulioside A, a main component of stevia, changed the population size of numerous genomes in the guts of mice. Consistent with this finding, an analysis of fecal matter from human volunteers revealed that rebulioside A decreased the growth of aerobic bacteria. Stevioloside, another main component of the sweetener, decreased the growth of anaerobic bacteria, which suggests that the consumption of stevia as a whole may disrupt bacterial communities. These reports can be used to make sense of stevia’s damaging impact on the lifespan of *D. melanogaster*, because microbial dysbiosis is associated with inflammation and, further, decreased longevity. In contrast to this explanation, however, stevia has been reported to increase the production of SCFAs, which have been correlated with healthful effects in humans. The sweetener has also been linked to anti-oxidant, anti-diabetic, anti-hypertensive, and anti-dyslipidemiac properties, which suggests, conflicting with the results of the experiment, that stevia would sustain the longevity of *D. melanogaster*.

Monk fruit caused a significant decrease in longevity compared to sucrose, although by smaller differences than ace-K and stevia, which is consistent with its reported anti-diabetic, anti-oxidant, anti-inflammatory, and anti-tumor attributes. Recent research has also advanced the sweetener as a probiotic, as altered mogrosides, monk fruit’s sweetening component, were found to increase populations of commensal bacteria in a lab setting. An increase in commensal bacteria would suggest salubrious impacts on health, and, therefore, longevity. However, this impact, though potentially beneficial, still qualifies as an alteration to the gut microbiome. Another explanation for monk fruit’s observed life-shortening impact may be found in the lack of conclusive research surrounding its genotoxicity.

The gut microbiome plays an important role in protecting the body against chronic inflammation, which is disadvantageous for healthy aging in all organisms. Dysbiosis can inhibit beneficial bacteria, damage microbial metabolism, and increase inflammatory mediators. Thus, the disruptive
interactions between ace-K, stevia, monk fruit, and the gut microbiome may serve as a primary explanation for each sweetener’s reductive impact on the survivorship of *D. melanogaster*. These sweeteners, therefore, may also have a deleterious impact on human longevity due to the conservation of metabolic and microbial processes between the organisms, which would warrant immediate scrutiny of their widespread use.5

Despite the significance of the results, the possibility that decreased survivorship relative to sucrose was due in part to *D. melanogaster*’s affinity for natural carbohydrates was a source of uncertainty.13 It is also important to acknowledge that the sufficient number of *D. melanogaster* pupae for the experiment (240 flies) did not all emerge on the same day, but instead over the course of two days, meaning that the flies may not have been perfectly age-synchronized. Additionally, due to differing molecular weights of each sweetener, the usage of the constant concentration of 50 g of sweetener per liter of solution resulted in varied molar strengths of each substitute, with ace-K being the most molecularly concentrated. The usage of a constant L concentration may have also been a source of error because the concentrations of each sweetener exceeded their ADIs due to the miniscule body weights of *D. melanogaster*.

To address these sources of error, future research should strive to subject organisms, separated by gender, to comparable conditions. This would include equal food and water access, as well as a constant concentration of sweetener. These amendments would allow for the comparison of results across studies and an understanding of how sweetener consumption impacts the gut microbiome. Ideally, research of this sort could build into human longitudinal studies that would correlate the consumption of NNS, gut microbial shifts, and longevity. Results should then be offered to the body of public knowledge about the interaction between dietary interventions and health aging.

Acknowledgements

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References

### Hypothesis:
If *Drosophila melanogaster* were exposed to 50 g/L of acesulfame-potassium, stevia extract, and monk fruit, then the consumption of acesulfame-potassium would result in the shortest lifespan. Acesulfame-potassium induces microbial dysbiosis, which is linked to inflammation and, further, decreased longevity.

### Independent Variable
The type of non-nutritive sweetener (ace-k, stevia, or monk fruit)

<table>
<thead>
<tr>
<th>Levels of Independent Variable</th>
<th>Sucrose (Control) (50 g/L)</th>
<th>Acesulfame-Potassium (50 g/L)</th>
<th>Stevia extract (50 g/L)</th>
<th>Monk fruit (50 g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of <em>Drosophila</em></td>
<td>60</td>
<td>60</td>
<td>60</td>
<td>60</td>
</tr>
</tbody>
</table>

### Dependent Variable:
Number of survivors (# of flies)

### Constants
- The approximate age of the flies when the experiment began (based on pupae emergence
- Groups of flies kept in the same environment (space, temperature, exposure, etc.)
- Pure extract of sugars (no added substances such as erythritol, maltodextrin, etc.)
- Concentration (g/L) of each non-nutritive sweetener
- Recordings taken at the same time
- Breed of flies (wild-type)
- Method/equation used to calculate deaths

### Control
Sucrose (traditionally used caloric sweetener/table sugar)

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**Figure 2**

*Survivorship means and standard deviations of sucrose, ace-K, stevia, and monk fruit diets*

Note. Figure 2 displays the mean survivorship of the sucrose, ace-K, stevia, and monk fruit diets on each day, in intervals of three to four days. Error bars represent variance in survivorship counts, with the positive side reflecting the maximum survivorship on a given day, and the negative side reflecting the minimum survivorship on that day.
**Table 1**  
Sucrose (control), ace-K, stevia, and monk fruit-fed *D. melanogaster* raw survivorship counts

<table>
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<th>Day #</th>
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<th>4</th>
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<th>11</th>
<th>15</th>
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<td>18</td>
<td>12</td>
<td>7</td>
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</table>

*Note. Table 2 displays the survivorship of *D. melanogaster* from the two vials used for sucrose, ace-K, stevia, and monk fruit. Each vial contained 15 male and 15 female *D. melanogaster*. Counts were taken every three to four days. Sucrose was used as the control.*
Table 2
Survivorship and Chi-Square results of D. melanogaster fed on sucrose, ace-K, stevia and monk fruit (N=240)

<table>
<thead>
<tr>
<th>Group</th>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Stevia</td>
<td>60</td>
<td>43</td>
<td>31</td>
<td>13</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Monk fruit</td>
<td>60</td>
<td>57</td>
<td>46</td>
<td>40</td>
<td>36</td>
<td>25</td>
<td>16</td>
<td>7</td>
<td>6</td>
</tr>
</tbody>
</table>

Note. Table 3 displays both raw data and Chi-Square analysis results. Total survivorship counts for sucrose, ace-K, stevia, and monk fruit between both vials of 30 D. melanogaster are listed on an interval of three to four days. For Chi-Square analysis, df=9, α=.05, and the critical value was 16.919. χ² values reflect the statistical difference of survivorship counts between each NNS (observed) and sucrose (expected).

*p < .00001

**p < .05

Figure 3
Total survivorship of Drosophila melanogaster fed on sucrose, ace-K, stevia, and monk fruit

Note. Figure 3 displays the survivorship of Drosophila melanogaster fed on sucrose (blue), ace-K (purple), stevia (yellow), and monk fruit (red) as descending lines over the course of 32 days. Each dot represents the survivorship corresponding to the day counts were taken.
Table 3
*Day 15 survivorship and Chi-Square results of D. melanogaster fed on sucrose (control), ace-K, stevia and monk fruit (N=240)*

<table>
<thead>
<tr>
<th>Group</th>
<th>Survivors</th>
<th>$\chi^2$ (6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>Ace-K</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Stevia</td>
<td>6</td>
<td>78.3*</td>
</tr>
<tr>
<td>Monk Fruit</td>
<td>36</td>
<td></td>
</tr>
</tbody>
</table>

Note. Table 4 displays both raw data from day 15 and Chi-Square analysis results. Total survivorship counts for sucrose, ace-K, stevia, and monk fruit are shown. For Chi-Square analyses, df=3, $\alpha=.05$, and the critical value was 6.0. $\chi^2=78.3$ is the Chi-Square statistic value and reflects the statistical difference of survivorship counts between each NNS and sucrose. $^*p < .00001$